MECHANISMS OF ALUMINUM TOLERANCE IN TRITICUM AESTIVUM L. (WHEAT). II. DIFFERENTIAL PH INDUCED BY SPRING CULTIVARS IN NUTRIENT SOLUTIONS[1]

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ABSTRACT

Twenty spring cultivars of *Triticum aestivum* L. (wheat) were grown in solution culture with and without aluminum (Al) (74 μM, 2.0 mg L^{-1}) for 14 days. Root growth and shoot growth were depressed in all cultivars grown with Al; however, symptoms of Al toxicity were most evident on roots. Based on a root tolerance index (RTI = weight of roots grown with Al/weight of roots grown without Al), cultivar tolerance ranged from 0.18 ± 0.02 to 0.85 ± 0.03. Aluminum-affected roots were relatively short, thick, and had numerous undeveloped laterals. Leaves of some cultivars showed chlorosis resembling iron deficiency and others showed purple stems typical of phosphate deficiency.

Plants of all cultivars induced a progressive decline in nutrient solution pH from the outset of the experiment until Days 10–14. After this initial period, some cultivars rapidly increased solution pH, while others continued to depress solution pH throughout the experiment. Cultivar tolerance expressed as the RTI was negatively correlated with the negative log of the mean hydrogen ion (H^+) concentration, the minimum pH, and the slope of the pH decline, each calculated from pH data collected during the first 9 days of the experimental period before any sharp rises in pH. The relationship between solution pH and Al tolerance was similar to that reported previously for winter cultivars of *T. aestivum,* suggesting that the Al tolerance of a given cultivar is a function of its ability to resist acidification of the nutrient solution and hence to limit the solubility and toxicity of Al.

TAYLOR AND FOY (1985) used 20 winter cultivars of *Triticum aestivum* L. (wheat) to test the hypothesis that plant-induced pH of the rhizosphere (as reflected by pH of nutrient solutions) determined cultivar tolerance to Al. A strong correlation between cultivar tolerance to Al and the rate and magnitude of pH decline induced in nutrient solutions suggested that Al tolerance resulted from the ability of Al-tolerant cultivars to resist acidification and maintain a relatively high pH in the rhizosphere.

The results of Foy and Fleming (1978, 1982) suggested that the relationship between high solution pH and tolerance to Al does not necessarily hold for spring cultivars of *T. aestivum.* In contrast to the pH patterns induced in solutions by the winter cultivars studied by Taylor and Foy (1985), Foy and Fleming (1978) found that the Al-tolerant cultivar, BH-1146, progressively increased solution pH from the beginning of the experimental period. Also, changes in pH induced by an Al-tolerant, spring cultivar (UC-44-111) and an Al-sensitive, spring cultivar (Anza) did not differ until after 10–12 days growth, presumably when NH_4^+ was depleted from nutrient solutions (Foy and Fleming, 1982). As suggested by Taylor and Foy (1985), pH changes occurring after NH_4^+ depletion may reflect the effects of differential tolerance to Al rather than the cause of differential tolerance.

The objectives of this study were to identify possible inadequacies of the plant-induced pH hypothesis and to further test the relationship between Al tolerance and plant-induced pH of nutrient solutions using 20 spring cultivars of *T. aestivum.*

MATERIALS AND METHODS—To facilitate comparison of results, methods used in this study were the same as those reported by Taylor and Foy (1985). Twenty spring cultivars of *T. aestivum* (Table 1) were selected to represent a known range of Al tolerance or because they were commonly used for wheat production in

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the United States in 1979 (Briggle et al., 1982). Seeds of each cultivar were germinated in aerated, deionized water for 24 hr; seedlings were grown on cheesecloth in deionized water for 2 days and allowed to elongate for 5 days on plexiglass support frames in dilute nutrient solutions containing the following: 1.27 Ca, 0.27 Mg, 3.32 NO_3^--N, and 0.24 NH_4^+-N in mM.

Twenty-four uniform, 9-day-old seedlings from each cultivar were mounted on the covers of 8-L polyethylene containers. Each container supported 12 plants in four bunches of 3 plants each. The nutrient solutions were a modification of that used by Foy et al. (1967) and contained the following nutrients: 3.71 NO_3^--N, 0.30 NH_4^+-N, 1.27 Ca, 0.75 K, 0.27 Mg, 0.12 SO_4^{2-}-S, 0.10 HPO_4^{2-}-P in mM, and 58.5 Cl, 53.9 Na, 17.9 Fe, 6.6 B, 2.4 Mn, 0.6 Zn, 0.2 Cu, and 0.1 Mo in μM. In contrast to Foy et al. (1967), we supplied Fe as FeHEDTA (ferric hydroxyethylethylenediaminetriacetate) prepared from equimolar amounts of $FeCl_3$ and Na_3HEDTA. Growth containers were covered to inhibit algal growth.

Plants were grown in a controlled environment room at 24 ± 1 C for a 16-hr light period and at 19 ± 1 C during darkness. Relative humidity was not controlled but averaged 51 ± 4% during the light period and 95 ± 6% during darkness. The growth room was illuminated by 84 fluorescent (1,500 mA) and 40 incandescent lamps (60 w) located 0.9 m above the plant bases. The level of photosynthetically active radiation (PAR) was 179 ± 13 μmol s^{-1} m^{-2} at plant base level and increased to 219 ± 18 μmol s^{-1} m^{-2} at the top of the leaf canopy (40 cm). Although not controlled, solution temperatures were 22 ± 1 C at the end of the light period and 20 ± 1 C at the end of darkness.

A randomized block, factorial design, with 20 cultivars, two treatments, and three replicates (120 containers) was used. Due to space constraints, replication was achieved in time (external replication). Control and Al treatments within each replicate were blocked together to minimize variation. Treatments included the control (no added Al) and 74 μM (2.0 mg L^{-1}) Al, supplied as $AlK(SO_4)_2 \cdot 12H_2O$. This level of Al was selected to provide adequate separation of cultivars on the basis of tolerance to Al (Foy et al., 1965; Foy and Fleming, 1982). The pH of nutrient solutions was adjusted initially to 4.5 with HCl or KOH and was measured daily.

After 14 days of treatment, plants were harvested, divided into roots and shoots, rinsed four times in distilled water, dried to constant weight at 60 C, and weighed. A root tolerance

TABLE 1. *Differential Al tolerance of 20 spring cultivars of* T. aestivum *grown in nutrient solutions as measured by the root tolerance index (RTI)*

Variety	Mean RTI ± SE
Olaf	0.85 ± 0.03a
Justine	0.84 ± 0.05a
UC-44-111	0.83 ± 0.02ab
Waldron	0.72 ± 0.02abc
Sawtell	0.70 ± 0.10abc
BH-1146	0.68 ± 0.14bcd
Inia-66	0.60 ± 0.08cde
Prodax	0.59 ± 0.03cdef
Fielder	0.58 ± 0.03cdef
Penjamo-62	0.57 ± 0.05cdef
Anza	0.52 ± 0.03defg
Nugaines	0.47 ± 0.02efgh
Sonora-63	0.43 ± 0.04fghi
Thatcher	0.38 ± 0.05ghij
Fortuna	0.35 ± 0.02hij
Daws	0.33 ± 0.05hijk
Selkirk	0.31 ± 0.04ijk
Henry	0.26 ± 0.01jk
Era	0.24 ± 0.02jk
Ward	0.18 ± 0.02k

Note: Data presented as the mean RTI ± standard error. Means followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

index (RTI) was calculated by dividing the root yield of plants grown with Al by the root yield of plants grown without Al. The ability of the plants to alter the pH of nutrient solutions was evaluated by the negative log of the mean H^+ concentration, the minimum pH, and the slope (rate) of the pH change, each determined from the first 9 days of the experiment before sharp rises in solution pH occurred. Contrary to previous results with winter cultivars of *T. aestivum,* several spring cultivars showed minor differences in the rate of pH decline between the Al and no Al treatments; hence, the nature of the pH decline was evaluated only for the Al treatments.

Statistical analyses of the data were performed using simple regression, Duncan's Multiple Range Test, and descriptive statistics available on the Statistical Analysis System (SAS) version 79.6 (SAS Institute, Inc., 1982). Significance was defined at the 95% confidence level.

RESULTS—The 20 spring cultivars of *T. aestivum* differed in their tolerance to Al. Root growth was depressed in all cultivars (Table 1, Fig. 1, 2), and symptoms of Al toxicity were most evident on roots. Aluminum-affected roots were relatively short, thick, and had numerous undeveloped laterals. Leaves of some cultivars showed chlorosis resembling iron de-

Fig. 1, 2.   **1.** Relative growth of five spring cultivars of *T. aestivum* grown without Al. Cultivars from left to right are Olaf, Fielder, Nugaines, Daws, Ward. **2.** Relative growth of five spring cultivars of *T. aestivum* grown with Al (74 μM, 2.0 mg L^{-1}). Cultivars from left to right are Olaf, Fielder, Nugaines, Daws, Ward.

ficiency and others showed purple stems typical of phosphate deficiency (Taylor and Foy, 1985; Foy and Brown, 1964).

Classified on the basis of the RTI, cultivar tolerance ranged from 0.85 ± 0.03 for the most tolerant cultivar (Olaf) to 0.18 ± 0.02 for the most sensitive cultivar (Ward) (Table 1). Plants of each cultivar induced a progressive decline in nutrient solution pH from the outset of the experiment until Days 10–14. After this initial period, some cultivars rapidly increased solution pH (Fig. 3), while others continued to depress solution pH throughout the experimental period. For each cultivar, the pH decline prior to the rapid rise in pH was explained by a linear model, although this could be misleading because pH itself is a logarithmic variable.

The relationship between pH of nutrient solutions and tolerance to Al was analyzed by simple regression with the RTI as the independent variable and the negative log of the mean H^+ concentration, the minimum pH, or the slope (rate) of the pH decline as dependent variables. With the three replicates combined, the RTI was significantly correlated with the

slope of the pH decline ($r^2 = 0.464, P < 0.0001$, Fig. 4), the negative log of the mean H^+ concentration ($r^2 = 0.436, P < 0.0001$), and the minimum pH ($r^2 = 0.432, P < 0.0001$) (Table 2). In each case, tolerance to Al was associated with the ability of the cultivar to resist acidification of nutrient solutions. The explained variance in each of the combined analyses was lower than the variance explained when Replicates 1 and 2 were analyzed individually. Replicate 3 showed unusually low r^2 values due to anomalous data points (Table 2). Good agreement was noted between replicates for the solution pH–Al tolerance relationship.

DISCUSSION — The relative tolerance rating of the cultivars was in agreement with a number of previous reports (Foy and Fleming, 1982; Camargo and Oliveira, 1981; Polle, Konzak and Kittrick, 1978; Moore, Kronstad and Metzger, 1976; Kerridge, Dawson and Moore, 1971; Mesdag and Slootmaker, 1969; Foy et al., 1965). Several exceptions did occur. For example, root growth of BH-1146 increased with exposure to Al, and this cultivar induced an increase in solution pH from the outset of
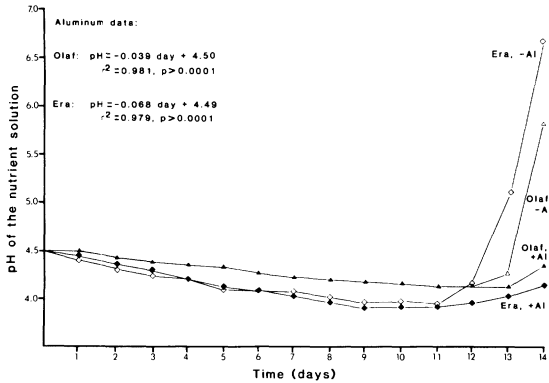
Fig. 3. Daily pH changes in nutrient solutions induced by an Al-tolerant cultivar, Olaf (triangles), and an Al-sensitive cultivar, Era (diamonds), of *T. aestivum* grown with Al (74 μM, 2.0 mg L^{-1}, solid symbols) and without Al (open symbols). Regression equations describe the relationship between pH of nutrient solutions and time for the first 9 days of the experimental period, prior to the pH rise.
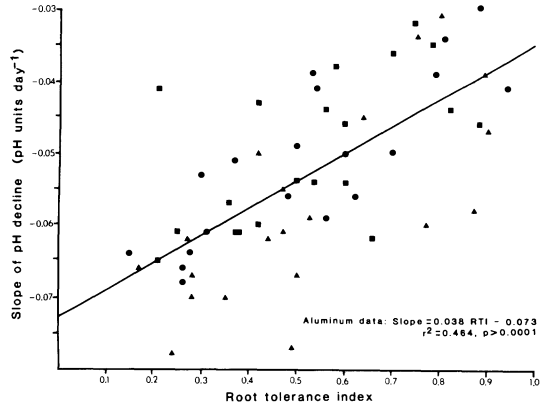

Fig. 4. Relationship between the root tolerance index (RTI) and the slope (rate) of the pH decline induced in nutrient solutions by 20 spring cultivars of *T. aestivum*. Replicate 1 = triangles; Replicate 2 = circles; Replicate 3 = squares.

an experiment conducted by Foy and Fleming (1982). In this study, BH-1146 was only moderately tolerant (RTI = 0.68 ± 0.14) and induced a progressive decline in solution pH. Our seed stock was not uniform, however, and a complete range of tolerance was noted in the BH-1146 plants grown with Al. Sonora-63 also exhibited variance within treatments, and the presence of some tolerant individuals resulted in a higher RTI value (0.43 ± 0.04) than expected, based upon the results of Foy and Fleming (1982). We have not been able to identify the cause of the variance within our seed stock of BH-1146 and Sonora-63.

Our classification of the cultivar Henry also differed from that of previous studies. Mesdag and Slootmaker (1969) classified Henry as intermediate in tolerance, while the results of this study showed it to be sensitive. Variation in the ranking and classification of cultivars reported in the literature indicates that screening for Al tolerance is imprecise. Such variation may have been due to factors like the extent of Al stress, screening techniques, composition of nutrient solutions, growth conditions, and seed source.

The daily patterns of change in nutrient solution pH induced by the spring cultivars of *T. aestivum* (Fig. 3) were similar to those reported by Taylor and Foy (1985), Fleming (1983), Foy and Fleming (1978, 1982), Mugwira, Elgawhary and Patel (1976), and Foy et al. (1965). However, the spring cultivars did not acidify nutrient solutions as rapidly as the winter cultivars studied by Taylor and Foy (1985). The less rapid decline in solution pH induced by the spring cultivars (as compared

with the pH induced by the winter cultivars studied by Taylor and Foy) was consistent with their greater tolerance to Al. While a winter cultivar (Atlas-66) showed the highest RTI of the two studies, only 4 of the 12 cultivars with an RTI less than 0.31 were spring cultivars. These cultivars were the most sensitive group, with means not significantly different according to Duncan's Multiple Range Test.

Confirming the results of Foy and Fleming (1982), the Al-tolerant cultivar, UC-44-111, and the Al-sensitive cultivar, Anza, did not induce different pH patterns prior to the rapid rise in nutrient solution pH. Nonetheless, the data for all 20 cultivars showed a significant correlation between the RTI and each of the pH variables. As with the winter cultivars of *T. aestivum* studied by Taylor and Foy (1985), cultivar tolerance to Al was correlated with the ability to resist acidification of the nutrient solution. Thus, it would appear that the lack of differential pH between UC-44-111 and Anza was not typical of the general relationship between nutrient solution pH and Al tolerance of *T. aestivum*.

The results reported here provide support for the hypothesis that plants that resist acidification of the rhizosphere and maintain a relatively high pH in the growth solution are exposed to a less Al-toxic rooting environment and exhibit greater tolerance to Al. Blamey, Edwards and Asher (1983) demonstrated that pH changes from 4.5 to 4.6 in dilute nutrient solutions resulted in a 26% decline in solution Al concentrations. Thus, the pH differences observed between the most Al-tolerant and Al-sensitive cultivars (in the order of 0.25 pH units on Day 9) in this study are likely of biological significance. Concurrent with the results of

TABLE 2. *Regression analyses between Al tolerance (RTI) and pH of nutrient solutions induced by 20 spring cultivars of* T. aestivum

Independent variable	Dependent variable	Replicate	Regression coefficient	Y-Intercept	r^2	Level of significance
RTI	Slope of pH decline	1	0.040 ± 0.009	-0.079 ± 0.005	0.502	0.0005
		2	0.042 ± 0.006	-0.073 ± 0.004	0.693	0.0001
		3	0.029 ± 0.010	-0.065 ± 0.006	0.320	0.0094
		1–3	0.038 ± 0.005	-0.073 ± 0.003	0.464	0.0001
	$-$Log mean H^+	1	0.28 ± 0.07	4.06 ± 0.04	0.473	0.0008
		2	0.22 ± 0.04	4.13 ± 0.02	0.599	0.0001
		3	0.18 ± 0.05	4.16 ± 0.03	0.368	0.0045
		1–3	0.23 ± 0.03	4.12 ± 0.02	0.436	0.0001
	Minimum pH	1	0.36 ± 0.07	3.78 ± 0.04	0.569	0.0001
		2	0.34 ± 0.06	3.89 ± 0.03	0.666	0.0001
		3	0.25 ± 0.08	3.94 ± 0.05	0.336	0.0074
		1–3	0.32 ± 0.05	3.87 ± 0.03	0.432	0.0001

Taylor and Foy (1985), the relationship between plant-induced pH and Al tolerance held for a broad range of Al tolerance. Solution pH accounted not only for the extremes in Al tolerance as demonstrated by Fleming (1983), Foy and Fleming (1978, 1982), Mugwira and Patel (1977), Mugwira et al. (1976), and Foy et al. (1965, 1967), but also for the Al tolerance of intermediate cultivars.

Despite previous data, which indicated that spring cultivars of *T. aestivum* may not conform to the plant-induced pH hypothesis (Foy and Fleming, 1978, 1982), the data presented here provided additional support for the role of plant-induced pH in determining tolerance to Al. These results, however, do not preclude the involvement of additional tolerance mechanisms in *T. aestivum*. Further studies may support the roles of cell wall complexation, exudation of chelate ligands, complexation of cytoplasmic Al by organic acids or metallothioneins, vacuolar sequestering of Al, alternate metabolic pathways, or the development of Al-tolerant enzymes as coincidental tolerance mechanisms.

LITERATURE CITED

BLAMEY, F. P. C., D. G. EDWARDS, AND C. J. ASHER. 1983. Effects of aluminum. OH:Al and P:Al molar ratios, and ionic strength on soybean root elongation in solution culture. Soil Sci. 136: 197–207.

BRIGGLE, L. W., S. L. STRAUSS, D. E. HAMILTON, AND G. H. HOWSE. 1982. Distribution of the varieties and classes of wheat in the United States in 1979. USDA, ARS, Stat. Bull. No. 676.

CAMARGO, C. E. DE, AND O. F. DE OLIVEIRA. 1981. Tolerancia de cultivares de trigo a diferentes niveis de aluminio em solucao nutritiva e no solo. Bragantia 40: 21–31.

FLEMING, A. L. 1983. Ammonium uptake by wheat varieties differing in Al tolerance. Agron. J. 75: 726–730.

FOY, C. D., AND J. C. BROWN. 1964. Toxic factors in

acid soils II. Differential aluminum tolerance of plant species. Soil Sci. Soc. Amer. Proc. 28: 27–32.

———, G. R. BURNS, J. C. BROWN, AND A. L. FLEMING. 1965. Differential aluminum tolerance of two wheat varieties associated with plant-induced pH changes around their roots. Soil Sci. Soc. Amer. Proc. 29: 64–67.

———, AND A. L. FLEMING. 1978. The physiology of plant tolerance to excess available aluminum and manganese in acid soils. *In* G. A. Jung, M. Stelly, D. M. Kral, and J. H. Nauseef [eds.], Crop tolerance to suboptimal land conditions, pp. 301–328. Amer. Soc. Agron., Crop Sci. Soc. Amer., Soil Sci. Soc. Amer.

———, AND ———. 1982. Aluminum tolerances of two wheat genotypes related to nitrate reductase activities. J. Plant Nutr. 5: 1313–1333.

———, ———, G. R. BURNS, AND W. H. ARMIGER. 1967. Characterization of differential aluminum tolerance among varieties of wheat and barley. Soil Sci. Soc. Amer. Proc. 31: 513–521.

KERRIDGE, P. C., M. D. DAWSON, AND D. P. MOORE. 1971. Separation and degrees of aluminum tolerance in wheat. Agron. J. 63: 586–591.

MESDAG, J., AND L. A. J. SLOOTMAKER. 1969. Classifying wheat varieties for tolerance to high soil acidity. Euphytica 18: 36–42.

MOORE, D. P., W. E. KRONSTAD, AND R. J. METZGER. 1976. Screening wheat for aluminum tolerance. *In* M. J. Wright and S. A. Ferrari [eds.], Plant adaptation to mineral stress in problem soils, pp. 287–295. Cornell University Agric. Exp. Stn., Ithaca, New York.

MUGWIRA, L. M., S. M. ELGAWHARY, AND K. I. PATEL. 1976. Differential tolerances of triticale, wheat, rye, and barley to aluminum in nutrient solution. Agron. J. 68: 782–786.

———, AND S. U. PATEL. 1977. Root zone pH changes and ion uptake imbalances by triticale, wheat and rye. Agron. J. 69: 719–722.

POLLE, E., C. F. KONZAK, AND J. A. KITTRICK. 1978. Visual detection of aluminum tolerance levels in wheat by hematoxylin staining of seedling roots. Crop Sci. 18: 823–827.

SAS INSTITUTE, INC. 1982. SAS user's guide: statistics. 1982 ed. SAS Institute, Inc., Cary, North Carolina.

TAYLOR, G. J., AND C. D. FOY. 1985. Mechanisms of aluminum tolerance in *Triticum aestivum* L. (wheat) I. Differential pH induced by winter cultivars in nutrient solutions. Amer. J. Bot. 72(5): 695–701.