

Aluminum toxicity in barley (*Hordeum vulgare* L.) root tips*

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ABSTRACT

The effect of aluminum (Al^{+3}) on cell division in barley (*Hordeum vulgare* L.) root tips was studied through hydroponic cultivation in a greenhouse. Cultivars FM 404 (tolerant) and PFC 8026 (susceptible) were submitted to 0, 2, 4, and 6 ppm of Al^{+3} for two, four, six, eight and 10 days. Statistical analysis showed that the inhibition of cell division caused by aluminum was significantly lower in the tolerant cultivar kept in 2 ppm for six and eight days. The regression analyses showed, for both cultivars, a highly significant variance of percentage of cell division (PCD) with relation to aluminum concentration, and a significant variance of PCD with relation to length of exposure. Regrowth of the material treated for eight days and subsequently transplanted to an aluminum-free solution for another two days revealed a higher PCD in the tolerant cultivar at the 4 and 6 ppm concentrations. The tolerant cultivar had better PCD than the susceptible cultivar with relation to aluminum toxicity.

INTRODUCTION

Barley is used in human nutrition, in animal rations, and, particularly in Brazil, in malting. The main areas where barley is grown in Brazil are the plateau of Rio Grande do Sul and the southern part of Paraná; the central areas of cerrado are deemed promising to the expansion of barley production. The soils of these areas are acid, a characteristic which in many cases is associated with low fertility (Silva, 1976; Brauner, 1982; Hutton, 1984). Under acid conditions, aluminum goes into solution and in excess inhibits the growth of plant roots (Foy *et al.*, 1965; Cavalcanti, 1990). Barley, among the winter crops, is the species most susceptible to aluminum toxicity (EMBRAPA/CNPT, 1989).

Toxicity due to aluminum, besides inhibiting growth, particularly root growth, interferes with absorption, transport and utilization of elements which are essential to the normal development of the plant, and it forms complexes with biomolecules (Malavolta *et al.*, 1981; Pavan, 1982; Horst *et al.*, 1983; Siegel and Haug, 1983; Wallace and Anderson, 1984). In barley plants, the main symptom of aluminum toxicity is a retarded root elongation (Foy *et al.*, 1967; Reid *et al.*, 1969, 1971; Ben and Comachio, 1983; Sandini, 1990). The harmful effects of aluminum in the root system of plants and cereals, such as wheat and barley, are possibly due to cellular and chromosomal damage (Buchholz and Foy, 1981; Moraes-Fernandes *et al.*, 1985; Zanella *et al.*, 1991) and cell division inhibition (Sampson *et al.*, 1965; Wallace and Anderson, 1984).

MATERIAL AND METHODS

The aluminum tolerant barley cultivar, FM 404, was selected from material of unknown origin (possibly

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from an Alpha cultivar - USA, 1921) by the brewery Companhia Cervejeira Brahma. An Aluminum susceptible barley cultivar, PFC 8026, was selected from cultivar TR 207 (Canada) by the National Center for Research on Wheat of the Brazilian Company of Research for Agriculture and Cattle Raising (CNPT/ EMBRAPA). Both cultivars were submitted to four solutions, each containing 0, 2, 4, and 6 ppm of Al^{+3} , respectively, in the form of $KAl(SO_4)_2 \cdot 12 H_2O$. These experimental solutions were placed in a system comprised of four gutters displayed parallel to each other in a greenhouse, each gutter containing Hoagland's solution with a specific Al^{+3} concentration. Hoagland's solution was modified in its phosphorus concentration, from 31 ppm to 0.62 ppm, to ensure that aluminum precipitation would not occur. Aeration of the solutions was obtained with the passing of air through PVC (Poly Vinyl Chloride) pipes placed at the bottom of the gutters. Air flow, kept unvaried by means of a compressor at a 1.5-in/cm² outflow, was forced out through minute holes, thus producing little air bubbles. The pH level was kept at 4.0 during the experiment. The pH level was controlled with the aid of a potentiometer and, when necessary, corrected twice a day at five different spots in each gutter.

Plantlets with both rootlet and leaflet, the latter being 1.5 cm long, were obtained after 60 h from germination of the seeds in germitest paper moistened with distilled water. These plantlets were placed on a grid mounted over the gutters so that their roots were immersed in the nutrient solution up to the crown. The plantlets were exposed to experimental solutions for two, four, six, eight, and 10 days. After each exposure, the root tips were collected between 9:30 a.m. and 10:30 a.m. and immediately fixed in acetic alcohol (3:1).

The root tips were used for counting those cells undergoing cell division, thus providing objective data for statistical analysis to verify the interference of aluminum in cell division. The root tips were hydrolized in HCl 5 N for 20 min at room temperature and subsequently immersed in Feulgen's solution for at least 45 min. On preparing the slides, the technique employed was squashing the material in 2% acetic orcein. The percentage of cells undergoing division (prophase, metaphase, anaphase, and telophase) was established for 500 cells per experimental unit.

The feasibility of the experiment demanded that each concentration was placed in a separate gutter. For each concentration (gutter) we had a factorial experiment with two factors, two cultivars, and five readings within the system of five piped blocks.

Individual statistical analyses were conducted for each concentration, according to the plan cited

above. When the variances of the residual of the four concentrations were seen to be homogeneous, the joint analysis of the four concentrations was conducted, according to the plan presented by Gomez and Gomez (1984).

Statistical analyses were performed through the Statistical Analysis for Microcomputers - SANEST (Zonta and Machado, 1984). The percentage of cell division (PCD) was estimated from the cell division frequency. Before the variance analysis, the data were transformed according to the arc sine transformation ($\text{arc sin } \sqrt{\text{PCD} / 100}$).

To establish the influence of regrowth on cell division, eight plantlets per gutter were rinsed in distilled water on the eighth day and transplanted to an aluminum-free nutrient solution. On the tenth day, the roots of these plantlets were harvested and fixed as described previously.

RESULTS AND DISCUSSION

The roots of the plants grown in the nutrient solution containing aluminum were short, thickened, lacked secondary ramifications, and had darkened spots. These symptoms were most evident in the susceptible cultivar, and were similar to the symptoms described for barley by Reid *et al.* (1969, 1971), Ben and Comachio (1983), Ben *et al.* (1988), and Sandini (1990). The analysis of variance for PCD revealed significant differences for levels of aluminum concentrations (1%), cultivars (5%), and interaction cultivar x length of exposure (1%).

For the tolerant cultivar FM 404, the analysis of polynomial regression for concentrations showed a linear regression of PCD following the increased concentration of Al^{+3} , significant at the probability level of 1% ($r^2 = 0.9201$). For the susceptible cultivar PFC 8026, the analysis showed a quadratic regression at the probability level of 1% ($r^2 = 0.9652$). The susceptible cultivar had a decrease of PCD up to the concentration of 4.56 ppm. The decrease in PCD, for the susceptible cultivar, however, was more dramatic than in the tolerant cultivar (Figure 1).

The quadratic regression can be explained by the few points used in the analysis. The point related to the higher concentration (6 ppm) is the one responsible for the quadratic model. Approximately 73% of the coefficient of determination, however, was due to the linear effect and 23% was due to the quadratic effect.

The highest PCD per concentration for the tolerant cultivar, when compared with the susceptible

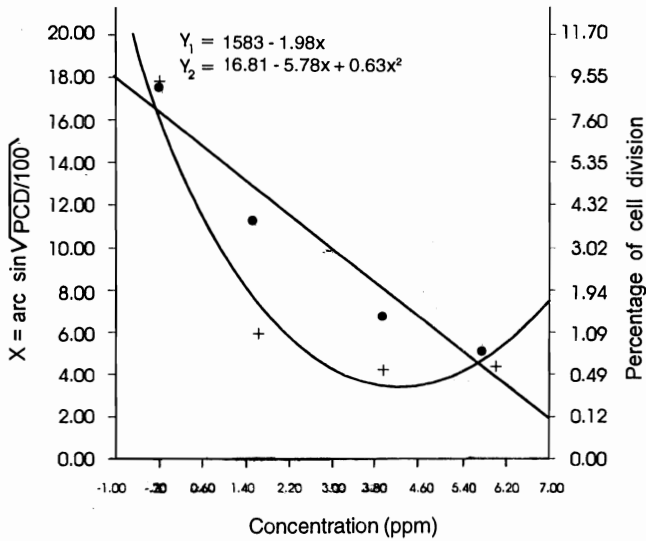


Figure 1 - Adjusted curve of percentage of cell division with relation to the effect of Al^{+3} concentrations (0, 2, 4 and 6 ppm) in barley plantlets of cultivars PFC 8026 (+) and FM 404 (•).

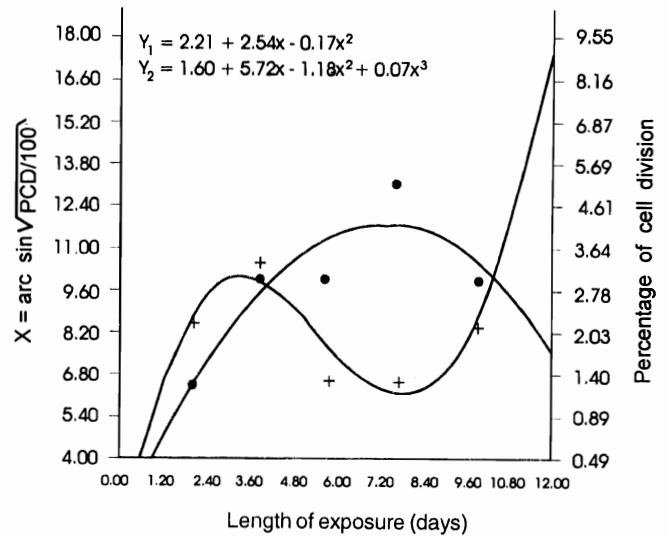


Figure 2 - Adjusted curve of percentage of cell division with relation to the effect of time of exposure to toxic Al^{+3} (two, four, six, eight, and 10 days) in barley plantlets of cultivars FM 404 (•) and PFC 8026 (+).

cultivar, corroborates the studies of Ben and Comachio (1983), Ben *et al.* (1988), and Sandini (1990), who reported that cultivar FM 404 was tolerant to aluminum toxicity and that cultivar PFC 8026 was susceptible to aluminum toxicity.

Although the interaction concentration x cultivar was not significant, the partitioning of its degrees of freedom is interesting from the biological point of view. Duncan's Multiple Range test (Gomez and Gomez, 1984) was used to compare the mean values of the cultivars at each level of the factor concentration, and the difference was highly significant between the PCD mean values of the tolerant and susceptible cultivars at the concentration of 2 ppm (3.45% and 1.28%, respectively). The difference in PCD values does not agree with Sandini (1990), who found the concentration of 4 ppm to be more effective for this differentiation. However, we emphasize that Sandini (1990) used the length of barley root as the parameter. According to Horst *et al.* (1983), inhibition of root elongation - the most sensitive parameter to study the toxic effects of aluminum - is basically the result of cellular inhibition of the root-tip meristems.

Analysis of the polynomial regression for the different lengths of exposure emphasizes the difference between the two cultivars. Cultivar FM 404 had a quadratic regression that was significant at the probability level of 1% ($r^2 = 0.8208$), with maximum PCD at 7.4 days. Cultivar PFC 8026 had a cubic regression, significant at the probability level of 5% ($r^2 = 0.8541$), with maximum PCD at 3.4 days and minimum PCD at 8.2 days (Figure 2).

It was also observed that a slight increase of PCD occurs upon the first contacts of the plantlets with the aluminum, which was also observed by Sampson *et al.* (1965) in barley roots. Initially, the effect of aluminum sulfate is presented as a small increase in PCD and, after 24 h, cells undergoing mitosis were absent.

The cubic behavior of PFC 8026 shows that approximately on the eighth day an increase occurs of the PCD. Horst *et al.* (1983) observed an increase in the frequency of cell division in *Vigna unguiculata* submitted to a continuous supply of aluminum. Those authors considered this recovery of the root meristems to be the plant's "adaptation in the short term".

For the Multiple Range interaction cultivar x length of exposure, Duncan's Multiple Range test was applied to the cultivars at each level of the factor length of exposure. The differences between the PCD mean values of cultivars FM 404 and PFC 8026 were significant for lengths of exposure of six and eight days, when the tolerant cultivar had its maximum PCD and the susceptible cultivar had its minimum PCD (Table I).

Toxic aluminum has an inhibiting effect on barley root-tip cell division. The cultivation of barley plantlets in nutrient solution with 2 ppm of Al^{+3} for eight days facilitates a higher level of differentiation (regarding PCD) between the tolerant and susceptible cultivars. The plantlets treated for eight days in the three concentrations of Al^{+3} and subsequently put in an aluminum-free nutrient solution for two days were evaluated for PCD as to whether they resumed growth. It was observed that, after the 10 days of cultivation to study regrowth, the tolerant cultivar had a PCD clearly

Table I - Means of percentage of cell division in barley root tip of cultivars FM 404 and PFC 8026.

Cultivars	Length of exposure (days)				
	2	4	6	8	10
FM 404 (Tolerant)	1.35a	2.95a	3.07a	4.97a	3.01a
PFC 8026 (Susceptible)	2.31a	3.27a	1.61b	1.47b	2.11a

These means followed by the same letter within the columns do not differ from one another, according to Duncan's test ($\alpha = 0.05$).

higher than the susceptible cultivar, as shown in Figure 3. At the highest concentrations of Al^{+3} , the tolerant cultivar resumed growth more effectively than the susceptible cultivar. Both cultivars had a stronger reaction to withdrawal of Al^{+3} when Al^{+3} was at a higher concentration. However, the tolerant cultivar maintained a PCD nearly twice that of the susceptible cultivar, which apparently suggests a lesser damage caused by Al^{+3} to the root meristem cells of FM 404.

Lopez-Cesati, cited by Sandini (1990), observed that the difference in regrowth reaction between wheat cultivars (tolerant and susceptible to Al^{+3}) has been used as a differential standard by the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT). This suggests that the regrowth reaction be verified as a parameter for identification of tolerant wheat material. The results of Sandini (1990) and of our paper support using regrowth as an evaluation test for barley cultivars tolerant to Al^{+3} , as it is already being done with wheat.

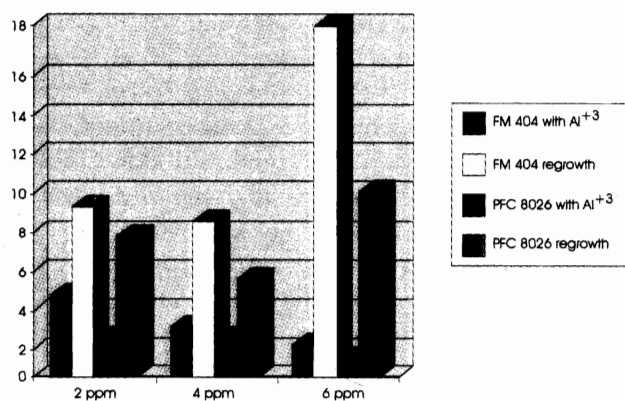


Figure 3 - Means of percentage of cell division in two cultivars (FM 404 and PFC 8026) for three different concentrations of Al^{+3} (2, 4 and 6 ppm) during both development with Al^{+3} for eight days plus two days with Al^{+3} and regrowth after the second day without Al^{+3} .

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RESUMO

Utilizando cultivo hidropônico, em casa de vegetação, estudou-se o efeito do alumínio (Al^{+3}) sobre a divisão celular em ponta de raiz de cevada (*Hordeum vulgare* L.). Foram utilizadas as cultivares FM 404 (tolerante) e PFC 8026 (sensível), submetidas a 0, 2, 4 e 6 ppm de Al^{+3} por dois, quatro, seis, oito e 10 dias de exposição. A análise estatística deste experimento, delineado no esquema fatorial em blocos casualizados, mostrou que a inibição da divisão celular, causada pelo alumínio, estimada pela percentagem de divisão celular (PDC), é significativamente menor na cultivar tolerante do que na sensível, na concentração de 2 ppm e nos tempos de exposição de seis e oito dias. As análises de regressão revelaram uma variação altamente significativa em relação às leituras, para ambas as cultivares. A observação da retomada de crescimento deste material submetido às condições acima descritas por oito dias e transplantado, após este período, para a solução de 0 ppm de Al^{+3} , por mais dois dias, mostrou que a PDC apresentou-se maior na cultivar tolerante do que na sensível, principalmente nas concentrações de 4 e 6 ppm. O presente trabalho mostrou que, de maneira geral, no que se refere à PDC, a cultivar tolerante apresenta um melhor desempenho que a sensível frente à toxidez por alumínio.

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