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Comparison of the response to aluminum toxicity in gametophyte and sporophyte of four tomato (*Lycopersicon esculentum* Mill.) cultivars

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Summary. We tested pollen from four tomato cultivars differing in sensitivity to aluminum in the sporophyte to determine if Al sensitivity was also expressed in pollen. Pollen sensitivity to Al was measured by the ability to germinate and grow in a control solution after a short period in a high concentration of Al. The response was ranked and compared to the Al sensitivity ranking of the four cultivars based on top growth in Al toxic soil. In addition, seedlings from the most and least sensitive cultivars, based on pollen germination, were compared for Al sensitivity in nutrient solutions. Treatment with Al significantly reduced pollen germination in the two more sensitive cultivars, but not in the two more resistant cultivars. However, the ranking was not the same as that based on the shoot growth of the sporophyte. Root growth as a criterion of sporophytic Al sensitivity produced results similar to pollen germination. The study suggests that although the correspondence is better for some phenotypic responses of the sporophyte than others, Al tolerance appears to be another character expressed in both pollen and sporophyte.

Key words: Aluminum tolerance – Tomato – *Lycopersicon esculentum* – Pollen

Introduction

About 60% of the structural genes expressed in the sporophytic stage of the plant life cycle are also expressed in pollen (Tanksley et al. 1981; Willing and Mascarenhas 1984; Sari-Gorla et al. 1986; Pedersen et al. 1987; Willing et al. 1988). Thus, it should be possible to use this considerable overlap in gene expression to select for desired sporophytic traits in pollen (Zamir 1983; Ottaviano and Muclahy 1989). Pollen provides a large, haploid population upon which to select and, once pollen with the desired characteristics is selected, recovery of plants, using the plant's sexual cycle, is straightforward.

Many of the genes expressed in both phases of the plant life cycle appear to relate to general metabolic function (Brewbaker 1971; Ottaviano et al. 1980; Weeden 1986). However, plant breeding objectives often involve qualitative traits such as disease resistance, cold or drought tolerance, and tolerance to mineral stress (Christiansen and Lewis 1982). Some of these characteristics appear to be expressed in pollen as well. For example, pollen sensitivities paralleling those of the parent plant have been reported for ozone (Feder 1986), salinity (Eisikowitch and Woodell 1975), temperature (Herrero and Johnson 1980; Zamir et al. 1982; Zamir and Vallejos 1983; Weaver et al. 1985), heavy metals (Searcy and Mulcahy 1985), and fungal toxins (Bino et al. 1988), but see Maisonneuve and Den Nijs (1984) for results in which there was no relationship.

Another stress of widespread economic importance, where one might anticipate an overlap in gene expression between pollen and sporophyte generations, is tolerance to aluminum (Al). Aluminum tolerance is based on nuclear genes (Lafever and Campbell 1978; Rhue et al. 1978) so that it could be selected for in pollen, and it tends to be specific for that metal (Foy et al. 1973a). Although aspects of Al tolerance such as patterns of accumulation in different tissues are related to whole plant architecture (Foy 1984), Al interferes with many functions that are found in both stages of the life cycle. For example, Al can interfere with mitosis (Clarkson 1969), interact with calmodulin (Haug 1984), and reduce cell wall extensibility and nutrient uptake (Foy et al. 1978). In vitro pollen tube growth (Konishi and Miyamoto 1983) and germination (Cox 1986) are sensitive to Al,

and in vitro cell culture studies (Meredith 1978a, b; Conner and Meredith 1985a, b) indicate that Al stress is one that can be studied and selected for at the cellular level.

To explore whether aluminum tolerance in the sporophyte was also expressed in pollen, we compared the Al sensitivity of pollen from four tomato cultivars. These cultivars were included in a study by Foy et al. (1973b) and differed in sensitivity to Al on the basis of top biomass measured after 50 days in Al-toxic Bladen soil, pH 4.2. Of the 18 cultivars included in their study, the cultivars chosen, 'Ace', 'Firesteel', 'Earliana', and 'Bonny Best' ranked 2, 8, 9, and 15 respectively, in order of increasing sensitivity to Al. In the present study, the pollen response to Al was compared to this ranking and to the sensitivity of seedlings from two of the cultivars, measured by comparing root growth in nutrient solutions with and without Al.

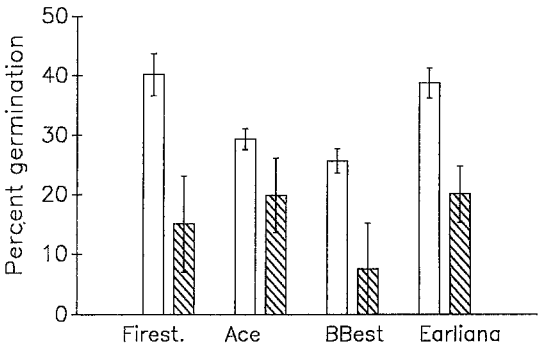

Fig. 1. Effect of pH on pollen germination. Percent germination at pH 5.5 (open bars) or at pH 4.4 (bars with diagonal lines). Data are the mean (arcsin) ±SE for three replicates made in fresh medium for each treatment and cultivar. Firest. = 'Firesteel', BBest = 'Bonny Best'

Materials and methods

The four cultivars of tomato, *Lycopersicon esculentum* Mill. – Ace, Firesteel, Earliana, and Bonny Best – were grown in standard potting soil in the greenhouse. Seed of Ace (Ace 55 VF) was obtained from the Burpee Seed Co. Seed of the other cultivars was obtained from Prof. R. Robinson, Cornell University and New York State Agricultural Extension Service, Geneva/NY.

Aluminum tolerance of pollen

Preliminary experiments indicated that pollen from these cultivars had low germination rates in the pH range used to test for Al toxicity. Therefore, the sensitivity of pollen to Al was measured by the ability to recover from a 30-min treatment with a high concentration of Al. This is similar to the cell rescue method of Connor and Meredith (1985b).

Pollen was pooled from several plants of the same cultivar, placed in 0.5-ml polypropylene vials, and hydrated in a humid chamber at 20 °C for 20 min. To test for Al sensitivity, hydrated pollen was suspended in 200 µl of germination medium (0.45 *M* sucrose, 1.62 m*M* H_3BO_3, 1.27 m*M* $Ca(NO_3)_2$, 500 µ*M* Al as $Al_2(SO_4)_3 \cdot 18\ H_2O$, 20 m*M* succinic acid-NaOH buffer in deionized, distilled water, pH 4.4). A control sample was suspended in the same medium without Al. After 30 min, the pollen suspensions were centrifuged, the original solutions were removed with a micropipet, and the pollen pellets were washed twice in germination medium at pH 5.5 without Al. Finally, the pollen pellets were resuspended in the germination medium at pH 5.5 and placed on a rotary shaker at 20 °C.

To test for the effect of low pH alone, pollen was suspended in germination medium at pH 4.4, and a control sample was suspended in germination medium at pH 5.5. The same procedure described above was followed, but following centrifugation the pollen pellets were resuspended in germination medium with the same pH as the original (pH 4.4 or 5.5). Initial concentrations of pollen were approximately 2 mg/ml, but since some pollen was lost during centrifugation and washing, pollen concentration probably varied from vial to vial. The experiments were stopped by placing the samples in a −20 °C freezer 2.5 h after the last resuspension.

Germination was scored on coded samples for 200 pollen grains and tube lengths measured for 25 pollen tubes in each sample. A pollen grain was considered germinated if the pollen tube was at least twice the diameter of the pollen grain, since stress may induce the extrusion of short pollen tubes (Stanley and Linskens 1974). For each cultivar, pollen germination and pollen tube length for the two controls and the Al-treated sample were compared with a one-way analysis of variance (Nie et al. 1975). The Tukey Kramer test was used to compare differences among the means. Comparisons between cultivars were done with a two-way analysis of variance (Nie et al. 1975). Pollen germination was low in some samples, so percent germination was transformed using the arcsin transformation prior to analysis (Sokal and Rohlf 1981).

Aluminum tolerance of sporophytes

Response to increasing Al concentration. Sixteen, 10-day-old, uniformly rooted seedlings of Earliana and Firesteel (the most and least sensitive to Al based on pollen germination) were placed through holes in a plastic foam tray and secured with a sponge plug. Each tray was floated on 5 l of Steinberg's solution (Foy et al. 1967) with 0, 38, 76, or 114 µ*M* Al as $Al_2(SO_4)_3 \cdot 18\ H_2O$. The pH of the solution was adjusted to about 4.6 prior to adding the Al to avoid precipitation. Trays were placed in a growth chamber with 16 h/28 °C days and 8 h/20 °C nights. The solutions were aerated continuously and changed on the fifth day. The pH was maintained at 4.6 ± 0.1 by daily additions of either HCl or NaOH. After ten days, plants were removed and the length of the first lateral root behind the growing tip of the longest root as well as the root system length of each plant were measured. Plants were then washed, divided into stem and roots, dried at 50 °C for 3 days, and weighed.

Recovery of root growth following a pulse of aluminum. In 1987, 20 cuttings of each variety were dipped in rootone, and when roots appeared, 10 cuttings from each cultivar were placed through holes in plastic foam trays and put in either 114 or 228 µ*M* Al in a modified Hoagland's solution, pH 4.5. The phosphate content in this solution was reduced to 0.1 m*M* (Conner and Meredith 1985b). After 48 h, the trays were removed, the plants were rinsed and then placed in 5 l of the same solution without Al. After an additional 5 days, regrowth of four roots from each plant was measured from the point where it had been inhibited in the Al-containing solution. (This region was discolored and constricted.) In 1988, a similar experiment was done with either 10- or 20-day-old seedlings using 171, 228, or 285 µ*M* Al in Steinberg's solution (Foy et al. 1967) at pH 4.6. Stems and

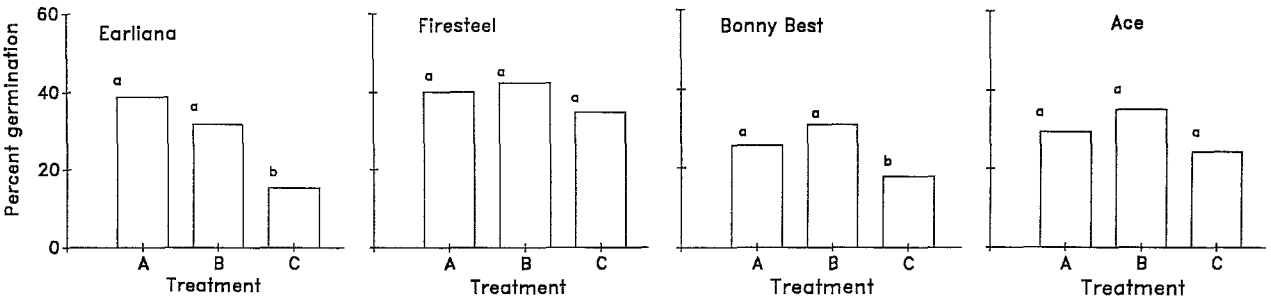

Fig. 2. Pollen germination following 30-min treatments in either control or Al-containing solutions. *A* Control, pH 5.5: initial suspension at pH 5.5 followed by resuspension at the same pH. (These data are the same as in Fig. 1). **B** Control, pH 4.4: 30 min at pH 4.4, followed by resuspension at pH 5.5. **C** Al-treated sample: 30 min in 500 μ*M* Al, pH 4.4 followed by resuspension at pH 5.5. Data are the means (arcsin) for 4 replicates for 'Firesteel', 7 for 'Ace, and 6 for 'Earliana' and 'Bonny Best'. Columns with a different letter within a cultivar are significantly different, $P=0.05$, using the Tukey Kramer test

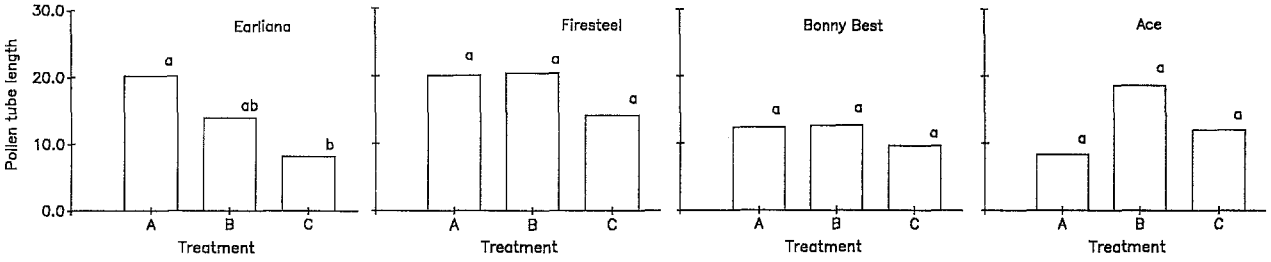

Fig. 3. Mean pollen tube length following 30-min treatments in either control or Al-containing solutions. Treatments and comparisons are the same as in Fig. 2. Pollen tube length is expressed as pollen diameters. Columns with a different letter within a cultivar are significantly different, $P=0.05$, using the Tukey Kramer test

roots were separated, dried at 50 °C for 3 days, and then weighed.

Data from both experiments were analyzed with a two-way analysis of variance (Wilkinson 1988). Data from the 1987 root regrowth experiment were log transformed prior to analysis. For the 1988 root regrowth experiment, differences among the means within each cultivar were compared with the Tukey Kramer test (Wilkinson 1988).

Results

pH sensitivity of pollen

The effect of pH alone on the germination of pollen from each of the four cultivars is shown in Fig. 1. Percent germination was reduced in all cultivars with reductions ranging from 31% of control values in Ace to 60% in Bonny Best. The reductions were significant for Firesteel ($t=3.32$, $df=3$, $P=0.045$) and Earliana ($t=3.49$ $df=4$, $P=0.025$). Except for Ace, which produced very short pollen tubes at both pH 5.5 and 4.4 (eight pollen diameters), the average pollen tube length was also reduced. Tube length was reduced to 36, 30, and 17 percent of control values for Firesteel, Earliana, and Bonney Best, respectively. Differences were highly significant for Earliana ($t=5.7$, $df=4$, $P=0.005$) and close to significant for Firesteel ($t=2.98$, $df=3$, $P=0.06$).

Aluminum tolerance of pollen

Comparison of pollen germination following 30 min in 500 μ*M* aluminum at pH 4.4 to germination in media without Al is shown in Fig. 2. Treatment for 30 min at pH 4.4 with no added Al followed by recovery at pH 5.5 had no significant effect on percent germination in any of the cultivars when compared to percent germination at pH 5.5 (treatment A vs treatment B). In fact, germination was slightly stimulated in three of the four cultivars. In contrast, a pulse of 500 μ*M* Al at pH 4.4, followed by a recovery period at pH 5.5 (treatment B vs treatment C), produced a highly significant reduction in percent germination in Bonny Best ($F=11.1$, $df=2$, $P=0.002$) and Earliana ($F=14.8$, $df=2$, $P=0.001$). These were the two more Al-sensitive cultivars in the study by Foy et al. (1973b) ranking 15 and 9, respectively. Reductions in percent germination were not significant for the more Al-resistant cultivars, Ace ($F=1.45$, $df=2$, $P=0.266$), ranked 2 by Foy et al. (1973b), or Firesteel ($F=3.58$, $df=2$, $P=0.08$), ranked 8. Compared to pollen germination following a pulse at pH 4.4 without added Al (treatment B vs treatment C), the cultivars can be ranked in the following order from least to most sensitive: Firesteel (82% of control), Ace (69% of control), Bonny Best (57% of control), and Earliana (48% of control).

Table 1. The effect of increasing Al concentration on length of lateral roots, root system length, and stem and root dry weight in 'Earliniana' (E) and 'Firesteel' (F). Data are the means of 14–16 plants of each cultivar. Within each column, means with the same letter are not significantly different at $P=0.05$ using the Tukey Kramer test

Al conc. (μM)	Lateral root (mm)		Root system (cm)		Stem dry wt. (mg)		Root dry wt. (mg)	
	E	F	E	F	E	F	E	F
0	32.5a	27.8a	14.1a	11.7a	16.7a	16.1a	4.24a	3.34a
38	32.1a	22.7ab	12.6a	11.2a	11.2b	12.2b	2.56bc	2.87ab
76	5.8b	13.4bc	11.9b	11.9a	8.2c	8.1c	2.95b	2.34b
114	4.9b	12.2c	11.3b	10.4a	8.4c	8.7c	1.94c	2.57b

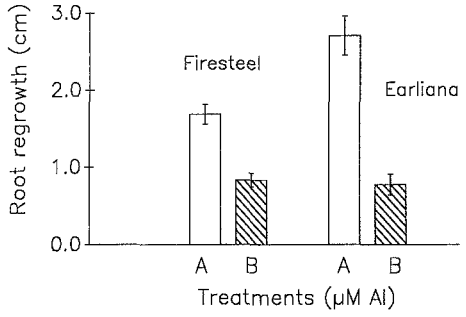

Fig. 4. Root regrowth measured 5 days after a 48-hour pulse in 114 (*A*) or 228 (*B*) μM Al in modified Hoagland's solution, pH 4.5. Measurements were made on cuttings dipped in rootone and which had started to root prior to testing. Means are the average of four roots from ten cuttings of each cultivar in each solution. Data are the means $\pm$SE

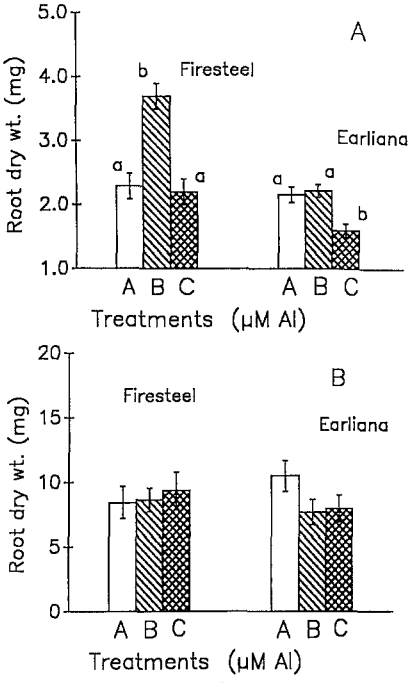

Fig. 5 A and B. Root dry weight measured 5 days after a 48-h pulse in 171 (*A*), 228 (*B*) or 285 (*C*) μM Al in Steinberg's solution, pH 4.6. Ten-day-old seedlings were used in **A** and 20 day old seedlings in **B**. The data are the means $\pm$SE. In **A** for each cultivar, columns with a different letter are significantly different, $P=0.05$, using the Tukey Kramer test. In **B** none of the means within a cultivar was significantly different

Mean pollen tube length following aluminum treatment and in controls is shown in Fig. 3. Mean pollen tube length did not appear to be as sensitive to Al as pollen germination. Mean pollen tube length was reduced compared to both controls by treatment with Al in three of the four cultivars. However, differences were significant only for Earliana. In Ace, there was a reduction compared to the pH control (pH 4.4, no Al, treatment B) but not for the control at pH 5.5 (treatment A).

A two-way analysis of variance comparing percent germination in Firesteel and Earliana indicated that these two cultivars differed in their response to Al (treatment $\times$ cultivar interaction, $F=4.42$, $df=2$, $P=0.026$). However, the mean pollen tube length of Firesteel and Earliana was not differentially affected by a pulse of Al (treatment $\times$ cultivar, $F=1.03$, $df=2$, $P=0.374$).

Aluminum tolerance of sporophyte

Increasing the concentration of Al significantly reduced the length of lateral roots and stem and root dry weight both in Earliana and Firesteel (Table 1). For root dry weight and the length of the lateral roots (Table 1), the reduction was significantly less for Firesteel (77% and 44% of control at 6 ppm Al, respectively) than for Earliana (46% and 15% of control at 6 ppm) (treatment $\times$ cultivar interactions: root dry weight, $F=4.87$, $df=3$, $P=0.003$; length of lateral root, $F=4.68$, $df=3$, $P=0.004$).

Measurements of root regrowth on cuttings following 48 h in 114 or 228 μM Al at pH 4.5 indicated that regrowth was less severely affected at 228 μM in Firesteel than in Earliana (Fig. 4). This difference was significant (treatment $\times$ cultivar interaction, $F=6.19$, $df=1$, $P=0.02$). In 1988, in a different nutrient solution and using seedlings rather than cuttings, the results were similar. Increasing the concentration of the pulse of Al produced a greater decrease in root dry weight in Earliana

than Firesteel (Fig. 5). The difference in root weight was significantly less at 285 than at 171 μM Al for Earliana when 10-day-old seedlings were used (Fig. 5A), but not when 20-day-old seedlings were used (Fig. 5B). There was also a significant interaction (treatment × cultivar, $F=8.27$, $df=2$, $P=0.001$) with the 10-day-old seedlings (Fig. 5A) but this was due to an increase in root weight of Firesteel at 228 μM Al. Differences in stem dry weight were not significant in either experiment.

Discussion

Percent germination in vitro of pollen from all four cultivars was reduced by a pH of 4.4 in standard germination medium. This is at the high end of the range of pH values that reduce pollen germination in a variety of broad leaved and coniferous plants (Cox 1986), so that tomato pollen appears to be quite sensitive to acid conditions. This sensitivity may reflect the general sensitivity of tomato sporophytes to a pH below 5 (Arnon and Johnson 1942; Islam et al. 1980). Nevertheless, pollen germination in vitro responded differently to a 30-min treatment in germination medium with a low pH compared to the same medium with Al. After a pulse at pH 4.4 alone, pollen from all cultivars germinated as well as the pH 5.5 control when placed in germination medium at pH 5.5. With Al added, percent germination was significantly reduced in the two cultivars that were most sensitive to Al. Thus, the difference between the cultivars in percent germination following treatment with Al probably reflects differential Al sensitivity rather than pH sensitivity. As was found for tolerance to copper and zinc (Searcy and Mulcahy 1985), mean pollen tube growth rate was less sensitive to Al than percent germination.

To be useful in screening or programs involving pollen selection, differences in the pollen response to Al should parallel the response of the parental cultivars. The differences in Al sensitivity based on pollen germination did not produce the same ranking as reported by Foy et al. (1973b) based on top growth in Al-toxic Bladen soil, pH 4.2. Although the more sensitive (Earliana and Bonny Best) and resistant (Ace and Firesteel) cultivars are identified by both methods, the details of the ranking differ. Firesteel and Earliana are adjacent in Foy's (1973b) ranking, but are the least and most sensitive when ranked by pollen germination. The ranking of the tomato cultivars in terms of reduction in percent germination at pH 4.4 (Fig. 1) is closer to the ranking reported by Foy et al. (1973b) since Ace is the most resistant, Bonny Best the least, while Firesteel and Earliana are intermediate. Thus, based on comparisons with top growth, the correspondence between Al sensitivity expressed by percent pollen germination and that of the sporophyte does not appear to be very strong. A lack of close correspondence between top growth and pollen germination has also been reported by Maisonneuve and Den Nijs (1984) in a number of cultivars of tomatoes for response to low temperature.

If other indicators of sporophytic tolerance to aluminum are used, the correspondence between the response of pollen and Al tolerance of tomato cultivars tested is closer. Part of the tolerance mechanism in tomato may be associated with differential uptake of Al (Foy 1984). In the study by Foy et al. (1973b), the two more resistant cultivars, Ace and Firesteel took up less Al (135 ppm) when compared to the more sensitive cultivars, Bonny Best (144 ppm) and Earliana (157 ppm). Although these differences in uptake were not significant, differences in uptake may help account for the pattern of pollen germination in which pollen from Earliana was the most affected.

Another important expression of aluminum toxicity is reduced root growth (Foy 1974; Horst et al. 1983). Comparisons of root growth in solutions with and without Al are one of the primary screening techniques for Al tolerance and generally show a good correspondence to performance in Al-toxic soils (Reid et al. 1971; Moore et al. 1976). When compared by root growth, the most and least sensitive cultivars of tomato based on pollen germination, Earliana and Firesteel, respectively, were both sensitive to Al, although the results were consistent with Firesteel being more resistant to Al than Earliana. Differences in root growth between Firesteel and Earliana were not statistically significant when older plants were used. This is similar to the results from several other studies in which young seedlings were more sensitive to Al toxicity than older ones (Thaworuwong and Van Diest 1975; Rengel and Robinson 1989).

All of the cultivars appeared to be more resistant to Al when the response was based on pollen germination than when based on root growth. A greater resistance to *Alternaria* toxin in pollen than observed in the sporophyte was also reported by Bino et al. (1988). As in their study, some of the difference might be due to the different lengths of time of exposure to the toxic substance, and in our study, due to separating the effects of pH and Al in the pollen part of the study but not when working with seedlings.

These results suggest that sensitivity to Al is another character that is expressed in both haploid and diploid generations. However, as has been found for cold tolerance (Zamir and Gadish 1987) or pollen selection for increased vigor in corn (Landi et al. 1989), one has to pick with care the particular phenotypic features of the sporophyte generation that correspond to the effect observed in pollen. To be successful in influencing the diploid generation, the component(s) of the response to the stress expressed in pollen should be an important part of the overall response. In the present study, after expso-

sure to Al, pollen germination in vitro corresponded most closely to Al uptake of the sporophyte and to various aspects of root growth. Close relationships between pollen tube growth rate, pollen germination, and root growth have also been found for several other systems: copper or zinc tolerance (Searcy and Mulcahy 1985), cold tolerance (Zamir and Gadish 1987), and sporophytic vigor (Ottaviano et al. 1982). Since reduced root growth is an important expression of Al toxicity, screening procedures using pollen could, therefore, be used to identify cultivars or individuals resistant to aluminum.

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